Reference: *Biol. Bull.*, **148**: 199–218. (April, 1975)

# REPRODUCTION OF THE EXTERNALLY BROODING SEA ANEMONE *EPIACTIS PROLIFERA* VERRILL, 1869 [1]

DAPHNE FAUTIN DUNN [2]

*Department of Zoology, University of California, Berkeley, California, and University of California Bodega Marine Laboratory, Bodega Bay, California*

Brooding in marine invertebrates may be defined as the retention of offspring by a parent through the embryonic stages usually passed in the plankton, thereby shortening or entirely eliminating the dispersal stage. Among sea anemones external brooding, in which the young are attached to or enfolded by the exterior surface of the parent, is an intriguing and uncommon phenomenon. Carlgren's (1949) catalog of actinians lists only 16 externally brooding species of an approximate world-wide total of 800. These species, unlike internally brooding anemones [in which ". . . the young develop, until after metamorphosis, within the parent's body" (Stephenson, 1928, p. 92)], are confined to polar and temperate seas, and at least five of them are entirely subtidal.

*Epiactis prolifera,* an actinian that broods its young on its lower column (Fig. 1), is "locally abundant" in the lower littoral zone in rocky parts of the Pacific coast of North America from Puget Sound to La Jolla (Ricketts and Calvin, 1968), thus providing a rare opportunity for research on this poorly understood mode of reproduction. Although it is the best known species in a genus containing 16 species (Carlgren, 1949), *E. prolifera* has been the subject of little detailed study. The major references to it are given by Hand (1955), who also provides the most complete morphological description of the animal.

Uchida (1934) and Uchida and Iwata (1954) discuss the anatomy and development of a Japanese actinian identified as *Epiactis prolifera,* but Uchida (personal communication) now believes it to be *Epiactis japonica.* The study by Uchida and Iwata (1954) is the only published account of the development of an externally brooding sea anemone. The literature contains speculative statements about the reproductive biology of *E. prolifera* (*e.g.,* Bovard and Osterud, 1918; Johnson and Snook, 1927; MacGinitie and MacGinitie, 1968; Ricketts and Calvin, 1968), but none is substantiated by published evidence. This study was done to enhance our knowledge of this fascinating species, and to extend our understanding of external brooding in marine invertebrates.

## Materials and Methods

During the period April 1970 through March 1972, 290 specimens of *Epiactis prolifera* were collected from the rocky open coast northwest of the University of

[1] This work was completed in partial fulfillment of the requirements for the Ph.D. degree in the Department of Zoology, University of California, Berkeley, and was supported by an NSF Traineeship.

[2] Present address: School of Biological Sciences, University of Malaya, Kuala Lumpur, Malaysia.

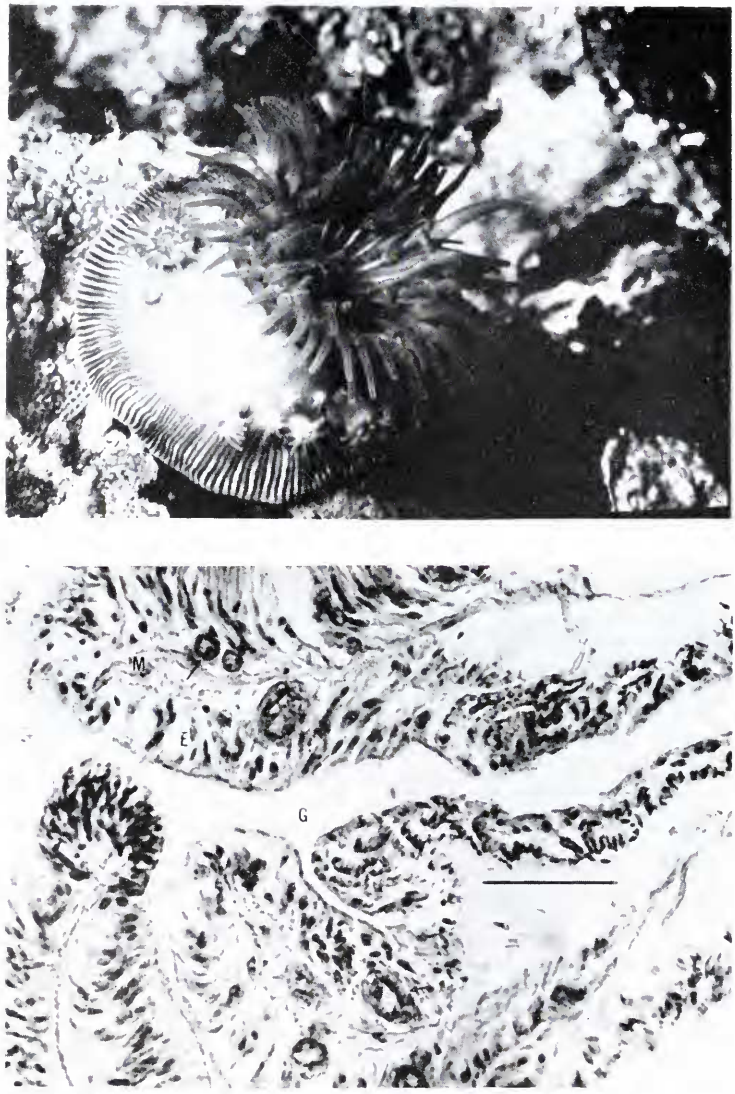


FIGURE 1. Expanded brooding *Epiactis prolifera*. Juveniles, which are attached low on the parent's column, are of various sizes. (Photograph by Robert Ames.)

FIGURE 2. Section showing oogonia (distinguishable by their large nuclei) in the endoderm beside the mesoglea of the mesenteries. Of the several oogonia visible, two are indicated by arrows; scale = 40 $\mu$. Labels indicate: M = mesoglea; E = endoderm; EC = ectoderm; G = gastrovascular cavity; O = oocyte; T = tubular channel of the trophonema.

California Bodega Marine Laboratory, Bodega Head, Sonoma County, California. In this locality *E. prolifera* generally occurs below the zero tide level although occasional individuals are found as high as a meter above datum. Thus observations and collections were confined to the monthly periods of extreme low ("spring")

tides, except when stormy weather or insufficiently low water prevented access to the animals altogether, and around the times of the spring and autumn equinoxes when the lower intertidal was exposed fortnightly.

Twenty-four collections of adult specimens of *E. prolifera* were made. (I term animals being brooded "juveniles" or "young" while non-brooded individuals are considered adults.) An effort was made to sample animals of a variety of sizes and colors from various sites and tidal levels, and with various numbers of young. Before an animal was removed from the substrate, its color, approximate vertical level, substrate, basal diameter, number of nearby specimens of *E. prolifera,* and number and size class of adherent young were recorded. To standardize conditions for measurement and color determination, each animal was tapped gently and a diving light was shone on it. The light also helped to reveal young. The edge of the brood groove was depressed when necessary to expose hidden juveniles. After prying the animals from the substrate (they come free quite easily with little or no damage), each was put in a plastic bag with an identifying tag.

Prior to fixation, which was done within 24 hours of collection, the anemones were relaxed with saturated chloral hydrate solution (four drops per 100 ml of sea water usually sufficed to make them unresponsive within an hour). The 9:11 menthol:chloral hydrate solution used by Chia and Rostron (1970) proved less satisfactory.

Several fixatives were tried. Bouin's fluid often penetrated *Epiactis* tissues incompletely and unequally, and, more seriously, caused the young anemones to fall off the adult. Zenker's fluid, and sea water formalin both preserved the young on the parent. Helly's fluid was also satisfactory. The approximate degree of shrinkage for each fixative was determined by comparing the basal diameter as measured prior to collection with the basal diameter of the anemone in the paraffin block prior to sectioning. Fixed diameter was 53% of live diameter with Zenker's ($N = 47$), 52% with formalin ($N = 47$), 82% with Bouin's ($N = 4$), and 56% with Helly's ($N = 9$). However, since the pedal disc is likely spread more broadly while still attached to the substrate than after collection, the calculated degrees of shrinkage are probably overestimates. It is also unlikely that all structures shrink equally, so shrinkage of 12 ova, the actual diameter of which were about 400 $\mu$, was calculated. Two Zenker's-fixed animals had recently spawned ova adhering to their bodies, the average diameter of the eggs on one being about 250 $\mu$, and on the other about 300 $\mu$. In another Zenker's-fixed *Epiactis,* freshly-spawned ova, averaging about 300 $\mu$ in diameter, had been ingested by attached juveniles. Thus the fixed diameters are about 62.5% and 75% of the actual dimensions. In a Bouin's-fixed anemone, eggs being spawned averaged about 250 $\mu$ across in section, approximately 62.5% of the actual diameter. Measurements in the following discussion, all made from fixed, sectioned material with an ocular micrometer, therefore represent 60–75% of the actual dimensions.

Routine staining was done with Ehrlich's hematoxylin and eosin Y, or phosphotungstic acid hematoxylin (Bowling, 1967). Trichrome stains were generally unsatisfactory. Histochemical tests included the Feulgen reaction, alcian blue-PAS, mucicarmine and Leach Bismarck brown (McManus and Mowry, 1960), the Wilson-Ezrin stain (Pearse, 1960), and toluidine blue and Mallory's triple connective tissue stain (Humason, 1967).

Microscopy was done with a Leitz Ortholux, and photomicrographs were taken with a Leitz Orthomat camera attachment using Panatomic X film.

## Results

### *Sexual character of* Epiactis

Gametes of *Epiactis prolifera* mature in the mesenteries between the retractor muscles and mesenterial filaments, as in other actinians. They occur in the lower half of the animal both above and below the opening of the actinopharynx into the coelenteron. The sexes of *Epiactis* are not separate, as has previously been reported (Cutress, 1949; Hand, 1955), but not all individuals exhibit hermaphroditism. The distribution of gonads (*i.e.*, gamete-bearing mesenteries) in anemones of various sizes, collected during the two years of this study, is shown in Table I.

Table I

*Distribution of gametes in animals of various sizes*

| Size class | Pedal disc diameter at the time of collection (mm) | Total N | Number sterile | Number female | Number hermaphroditic |
|---|---|---|---|---|---|
| 1 | up to 5.5 | 9 | 9 | 0 | 0 |
| 2 | 5.6–8.0 | 31 | 14 | 17 | 0 |
| 3 | 8.1–10.5 | 20 | 3 | 16 | 1 |
| 4 | 10.6–13.0 | 35 | 3 | 27 | 5 |
| 5 | 13.1–15.5 | 37 | 2 | 26 | 9 |
| 6 | 15.6–18.0 | 44 | 0 | 22 | 22 |
| 7 | 18.1–20.5 | 39 | 0 | 20 | 19 |
| 8 | 20.6–23.0 | 26 | 0 | 6 | 20 |
| 9 | 23.1–25.5 | 14 | 0 | 3 | 11 |
| 10 | 25.6 and up | 14 | 0 | 1 | 13 |
| | | 269 | 31 | 138 | 100 |

All animals with basal diameter less than 5.8 mm were without gonads, and none larger than 15.0 mm was sterile. The largest specimen examined was 35.7 mm.

In hermaphroditic individuals, gametes of the two sexes, in all stages of development, may occur in one mesentery. There is no vertical separation of spermatogenic and oogenic tissue. Sperm follicles and female gametes may alternate randomly in a single mesentery. Some mesenteries contain only the latter and, rarely, some have only sperm. Very early stages of both types of gametes often occur in the most peripheral part of the gametogenic area.

### *Arrangement of gametogenic mesenteries*

Primary and secondary mesenteries are always complete and sterile in adults (I found one anomalous fertile secondary mesentery). Normally only mesenteries of the third and fourth orders are gametogenic, but not all of them in a single anemone are fertile. Fifth order mesenteries, which appear only in larger animals and are generally confined to the basal region, are always sterile. Although the

mesenteries are usually regularly arranged through the fourth cycle, the 48 pairs of the fifth order are not added simultaneously. I never found an *Epiactis* with more than 96 pairs of mesenteries.

There is no apparent seasonal gonadal cycle, for animals with gametes in all stages of development are found throughout the year, and sexual state correlates with neither color nor intertidal height at the time of collection, but is related to size. However, changes in sexual state do not take place at a particular size in all animals. This may be partly because size is changeable and difficult to determine accurately in actinians. A parameter closely related to size, the number of mesentery pairs, can be more easily measured, is not subject to change over short periods, and correlates well with sexual state.

Very few anemones with less than four complete cycles of mesenteries contained oogonia or oocytes (the minimum number was 64 in a fertile individual). The proportions of fertile animals in size class 2 with fewer than 48 pairs, and with 48 or more pairs are significantly different. Size class 3 exhibits the same phenomenon less strongly. Oogonia and oocytes of these smallest fertile animals were sometimes in third, sometimes in fourth order mesenteries and, in purely female animals of all sizes, the number of fertile mesenteries of these two cycles were proportionately equal.

The smallest animal with sperm had a basal diameter of 10.1 mm. In size classes 4 and 5, the presence of male gametes was highly correlated with the existence of half or more of the fifth cycle of mesenteries. This correlation also held for size class 6, but not as strongly, and disappeared in size class 7. The one individual in size class 10 lacking sperm had as many mesenteries as the other 13 that were hermaphroditic.

In hermaphrodites, nearly four times as many fourth as third order mesenteries contained ova although there are only twice as many of the former as of the latter, and among the few mesenteries with only sperm, tertiary ones predominated over fourth order ones. There were proportionately equal numbers of third and fourth order hermaphroditic mesenteries.

### *Fertility and brooding*

Many of the animals not brooding at the time of collection were fertile, but all of those brooding young were sexually mature females or hermaphrodites. The smallest fertile adult collected was 5.8 mm in basal diameter, although one precocious adherent juvenile, 2.1 mm in diameter as measured in section, had oogonia and small oocytes. The smallest brooding animal collected was 7.8 mm, from the population of tan *Epiactis* on and around the alga *Cystoseira osmundacea* (Dunn, 1972), but the smallest one with young from coralline algae-encrusted rock was 11.6 mm.

### *Oogenesis*

Anthozoan germ cells arise in the endoderm, presumably from undifferentiated interstitial cells, but mature in the mesoglea of the mesenteries (Hyman, 1940). Figure 2 illustrates the earliest stage at which gonocytes were distinguishable in *E. prolifera,* as cells with relatively huge nuclei. They are small compared to the size

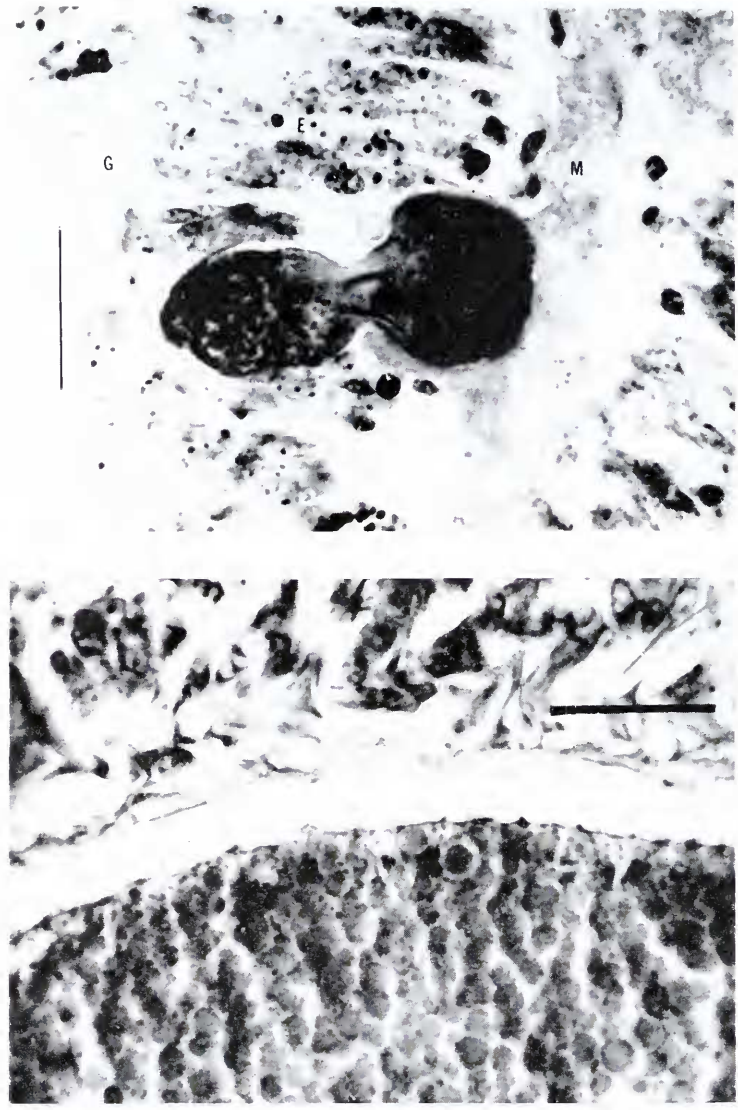


FIGURE 3. Oogonium being thrust from the endoderm into the mesoglea at cytokinesis. The mesoglea appears to bulge out to engulf the egg cell; scale = 20 $\mu$. For labeling, see caption to Figure 2.

FIGURE 4. Section of the edge of a yolky oocyte. Yolk granules are distributed uniformly throughout the cell. Spines on the membrane may be fixation artifacts; scale = 20 $\mu$.

they eventually attain as ova, but are considerably larger than somatic cells. Those in Figure 2 are probably oogonia, although early spermatogonia appear identical. Primary oogonia, ranging in diameter from about 6.0 to 8.5 $\mu$, grow and multiply in the endoderm. Gametes in the endoderm up to 30–35 $\mu$ in diameter are secondary

oogonia (with nuclei about half the diameter of the cell). These cells move from the endoderm into the mesoglea either by ameboid movement or at division, when one daughter cell is thrust into the mesoglea which appears to bulge to surround the oocyte, leaving the other daughter cell in the endoderm (Fig. 3). Female germ cells usually enter the mesoglea at a diameter of 20–25 $\mu$, and once there cease dividing, indicating that they have become oocytes. As an oocyte grows, the relative diameter of its nucleus changes from half or more that of the entire cell to a quarter or less (the actual size increases somewhat), and concomitantly there is an alteration in the cytoplasm's staining properties reflecting the onset of vitellogenesis. Yolk forms as platelets that are evenly distributed throughout the oocyte, as shown in Figure 4, which also depicts what appear to be spines 1.5–4 $\mu$ long on the egg membrane. These are visible from about the time that yolk formation begins, but might be fixation artifacts. The yolk granules concentrate around the germinal vesicle which moves, as the cell grows, from a central to a peripheral position. The germinal vesicle contains a single nucleolus at this stage and is Feulgen-negative. Germinal vesicles of oocytes in the same mesentery and at about the same stage of development exhibit no perferred orientation.

When the oocyte is 30–35 $\mu$ in diameter, a bulbous structure becomes apparent between it and the edge of the mesentery (Fig. 5). This soon elongates, forming a hollow tube extending from the edge of the mesentery, penetrating the mesoglea, and indenting the egg cell (Fig. 6). It is 15–20 $\mu$ in total diameter, and appears to be identical to a structure first described by Hertwig and Hertwig (1879) from *Sagartia* (now *Calliactis*) *parasitica,* and called by Hertwig (1882) the "filamental apparatus." Hertwig (1882) found it in four other actinian species, and Nyholm (1943) described the same structure from *Cerianthus lloydii,* calling it the "trophonema." I shall use this term for it. In oocytes in which vitellogenesis has begun, the germinal vesicle is located on the side of the oocyte nearest the trophonema. Where it reaches the oocyte, the trophonema flares out, due to the inflated ends of the several cells that comprise the structure and surround its tubular channel. In very large oocytes these cells become considerably flattened (Fig. 7) so that the tubular interior is apparently occluded, and the structure may even seem to disappear.

When full-grown, female gametes break through the thin layer of encapsulating mesoglea and thicker endoderm into the gastrovascular cavity (Fig. 8), whence they travel up the throat and out the mouth.

### *Spermatogenesis*

The first point at which male gametes are unmistakably identifiable is when bundles of 32–64 spermatogonia, each cell averaging 5.5 $\mu$ in diameter, are already enveloped by mesoglea. Layering becomes obvious as spermatocytes, average diameter 3.5 $\mu$, come to lie central to the spermatogonia, and as the cells differentiate further, spermatids (1.1–1.3 $\mu$ in diameter) occupy the center of the follicle. Finally, in mature follicles, spermatozoa, with apparently conical heads and an average diameter of about 0.7–0.8 $\mu$, fill the center (Fig. 9). Their tails converge toward the edge of the mesentery which will break to release them.

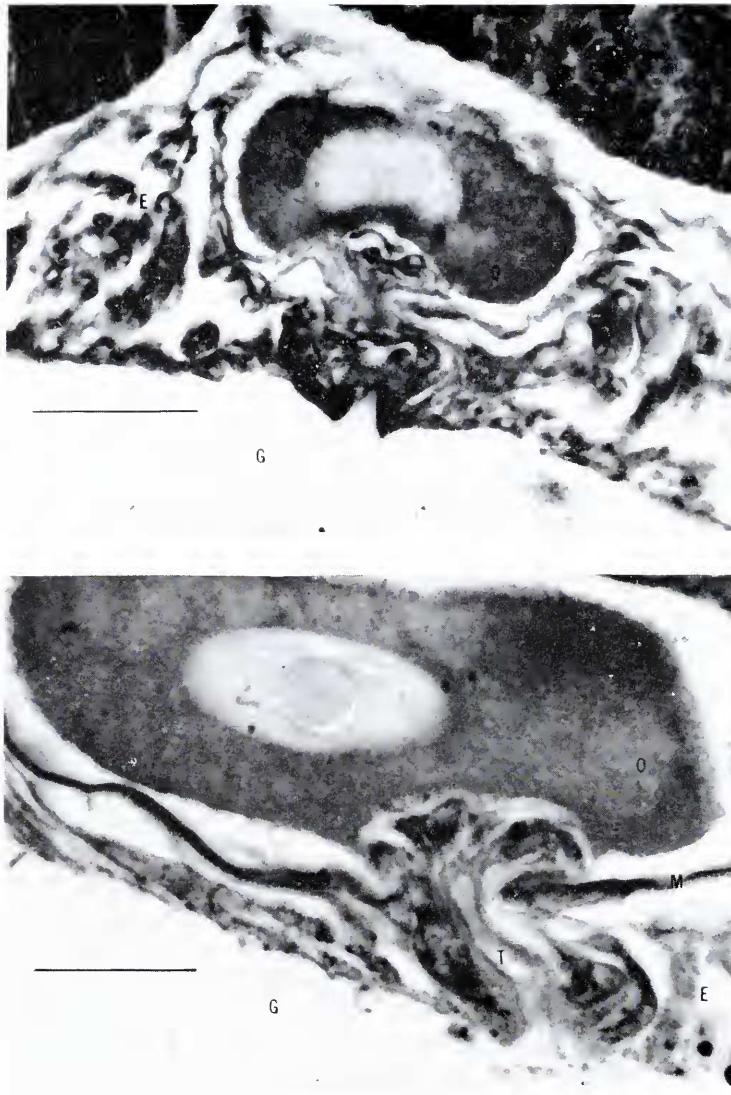


FIGURE 5. Section of a bulbous trophonema between early oocyte and mesentery edge; scale = 20 $\mu$. For labeling, see caption to Figure 2.

FIGURE 6. Indentation of a more mature oocyte by the trophonema. Note that it penetrates the mesoglea and that it has a hollow, tubular interior; scale = 20 $\mu$. For labeling, see caption to Figure 2.

*Spawning*

On 9 June 1971, I collected two specimens of *Epiactis* from the rocky intertidal for laboratory observation. The next day I noticed in the field that a number of animals were spawning or had done so since the previous day. When I returned

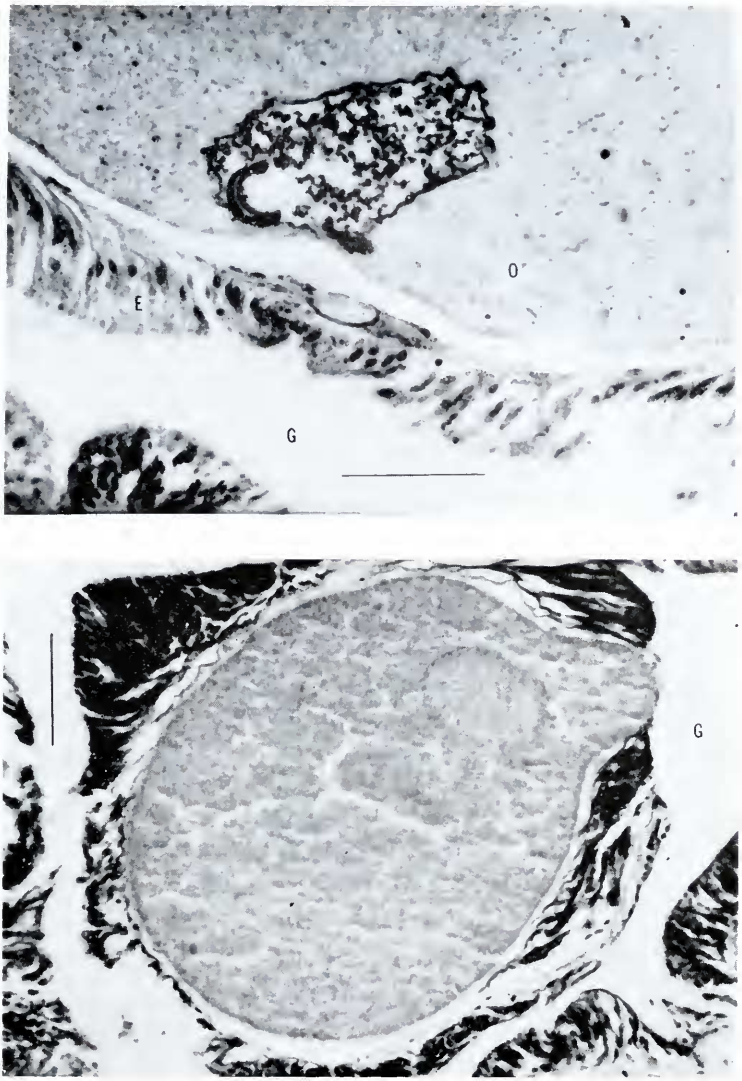


FIGURE 7. Section through a large oocyte with flattened trophonema. The tube appears to have closed by this time; scale = 40 $\mu$. For labeling, see caption to Figure 2.

FIGURE 8. Oocyte breaking through the tissues of the mesentery into the gastrovascular cavity; scale = 50 $\mu$. For labeling, see caption to Figure 2.

to the laboratory, one of the two individuals that I had collected was also spawning. The following day none of the several hundred animals that I carefully examined in the field was spawning, but the second anemone in the laboratory had a number of new spheres attached to its column. I also observed mass spawning in the field and laboratory on 15 and 16 October 1970. In all cases the sexual products expelled were pink or pinkish-orange spheres about 400 $\mu$ in diameter. I was un-

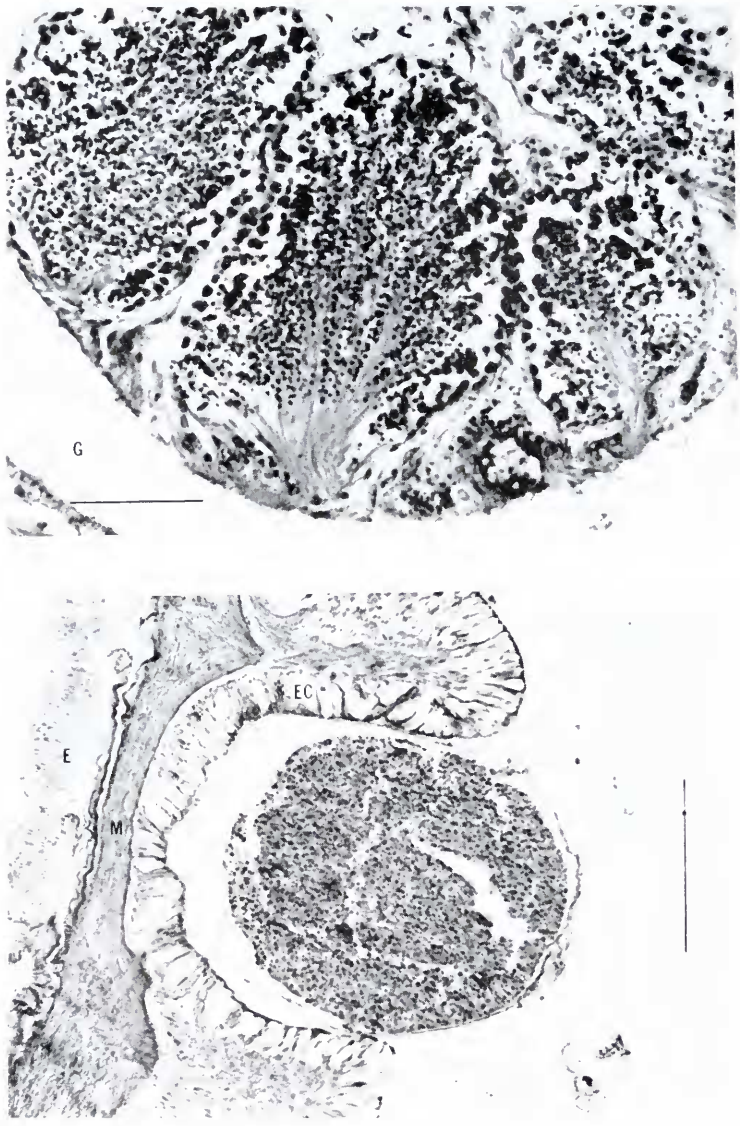


FIGURE 9. Section of a mesentery containing mature sperm follicles. Note the less mature cells peripherally and that the tails of the spermatozoa converge toward the edge of the mesentery; scale = 40 $\mu$. For labeling, see caption to Figure 2.

FIGURE 10. Section of a recently-spawned sphere attached to its parent's body wall. Notice that the yolk has shrunk inside the egg membrane which adheres tightly to the parent ectoderm; scale = 150 $\mu$. For labeling, see caption to Figure 2.

able to determine whether they were ova or zygotes. I never observed the release of sperm.

When I first noticed it in the laboratory, the spawning individual had about 25 spheres, enveloped in a common mass of mucus, on its lips. As the animal (18.7

mm basal diameter and completely covered with water) extended its column and opened and closed its mouth, with concomitant bulging and constriction of the throat and lips, the bundle of spheres twisted, then finally tumbled from the lips to the edge of the oral disc. This process lasted about 10 minutes. More spheres, visible inside the throat when the mouth opened, were gradually expelled a few at a time, ascending not in a siphonoglyph but at the sides of the actinopharynx. At the end of an hour about 50 had been spawned and were resting on one side of the oral disc. These eventually drifted down over the edge of the disc and some of them adhered to the column. During the next several days no development was discernible in either the spheres that had attached or those that had not. The day after spawning the spheres appeared more firmly adherent to the parent ectoderm than they had been originally, but nine days later they had disappeared.

*Embryology*

Figure 10 shows a section of an attached, recently spawned sphere. Although the parent body wall does not form an actual brood-pit, the ectoderm is thinned to about half its normal thickness beneath the sphere and thickened around it. The mesoglea is also somewhat thinned, but the endoderm is unaffected.

In an *Epiactis* zygote, the nucleus divides many times before the cytoplasm does, and the daughter nuclei segregate into two distinct layers, foreshadowing the body layers. There is never a blastocoel. Cytokinesis then occurs, resulting in ectodermal cells with nuclei at their distal ends, and endodermal cells with central nuclei (Fig. 11), producing what can only be termed a gastrula. Its high yolk content makes the inner layer considerably thicker than the ectoderm, which is soon devoid of yolk altogether. Mesenteries then form in the yolky interior. The embryo is never ciliated. Tentacle buds arise next, and finally a tiny *Epiactis*, gastrovascular cavity still mostly filled with yolk, is produced (Fig. 12). By the six-mesentery-pair stage most of the yolk is gone, but even before it has been completely absorbed, the juvenile has fully developed nematocysts, spirocysts, and gland cells in its tentacles (Fig. 13). These are functional, and juveniles can capture small organisms (Dunn, 1972, p. 106). The size of the contracted juvenile at this stage is not much greater than that of the zygote.

The ectoderms of parent and juvenile are closely apposed, and the cells of the two are, in material that has not been badly disrupted by histological processing, almost indistinguishable at some points. However, when this intimate relationship is disturbed, a strip of mucoid material is visible between the two animals (Fig. 14). Figure 15 shows an *Epiactis* embryo that has come loose from its parent, with a band of mucus extending from the parent over the embryo. Two types of large and numerous gland cells are present in the parent ectoderm, but there are none in the juvenile.

## Discussion

The sexual phenomenon exhibited by *Epiactis prolifera*, wherein the population consists of females and hermaphrodites but no males, is termed gynodioecy (Mather, 1940). In *Epiactis* it resembles protogyny, but there is never complete sexual reversal. Brooding animals are frequently hermaphroditic, as are many sedentary species (Ghiselin, 1969). *E. prolifera* is thus another example of a brooding, sedentary hermaphrodite.

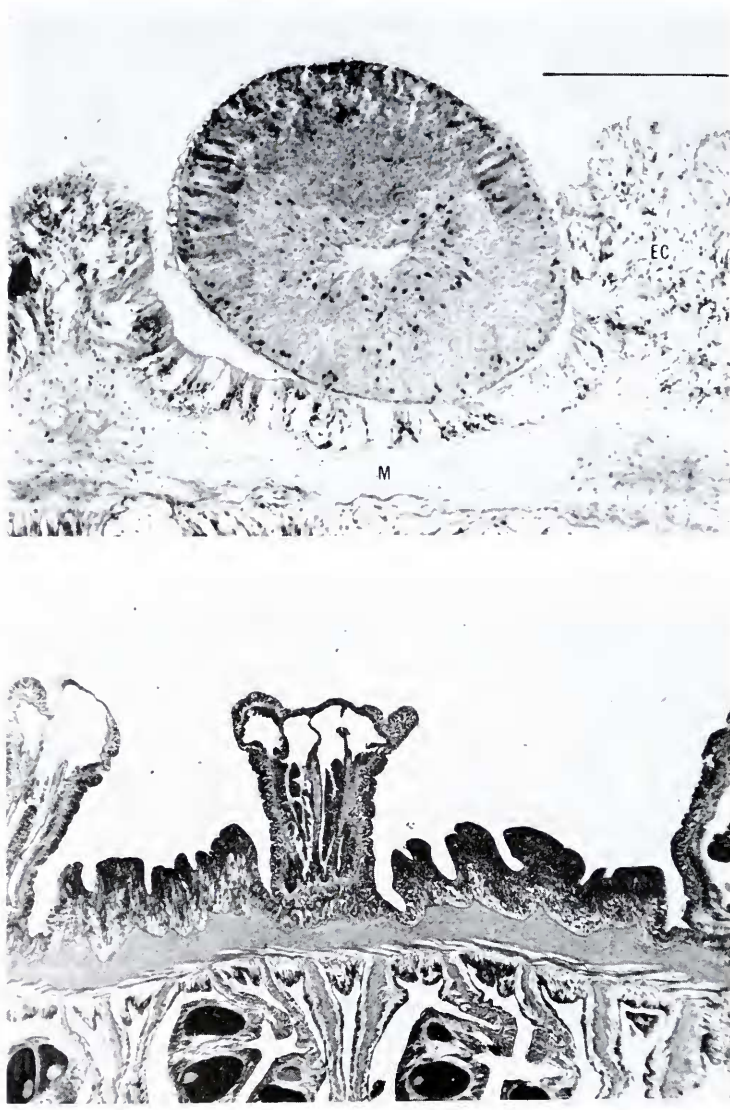


FIGURE 11. Section of an adherent gastrula. Its endoderm is thicker than its ectoderm because of the former's high yolk content; scale = 150 $\mu$. For labeling, see caption to Figure 2.

FIGURE 12. Section through an adult with adherent small juveniles, which are about 2 mm in diameter at this stage.

Earlier reports that *E. prolifera* is dioecious might have resulted from examination of few and/or small individuals. Perhaps, too, gonads were not examined in section so that only larger oocytes, visible to the naked eye, were seen. Even under a dissecting microscope, I had difficulty discerning oogonia, small oocytes and sperm follicles. Large samples of sea anemones of different sizes, collected at various

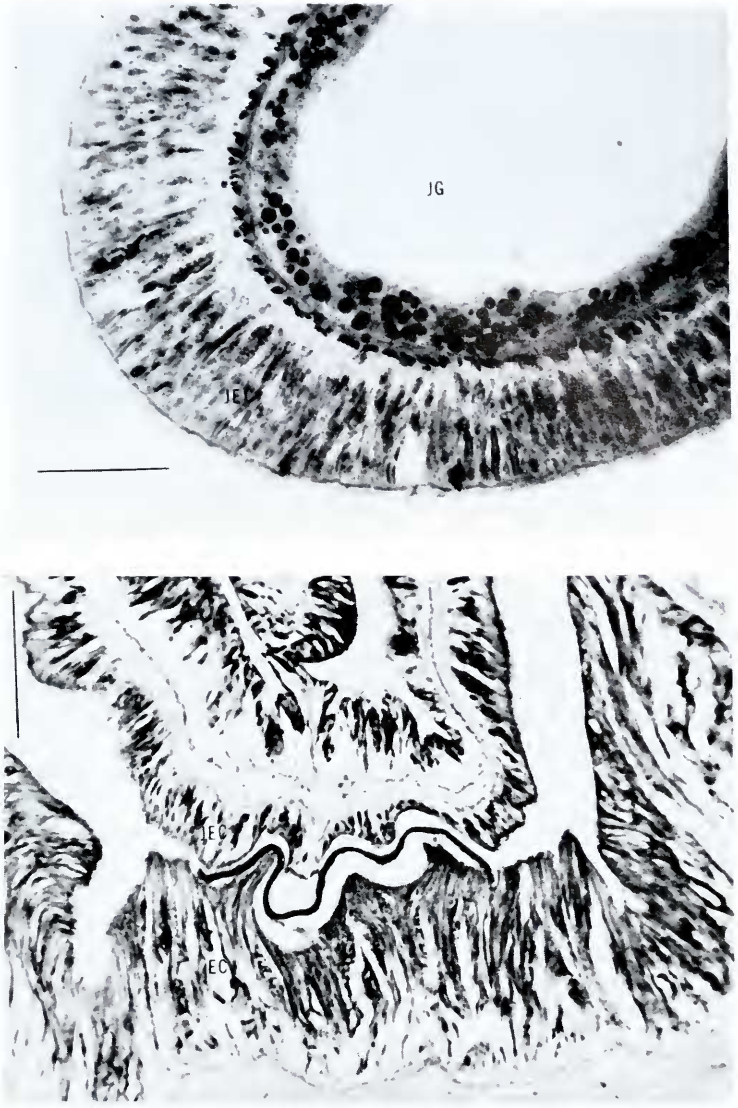


FIGURE 13. Section of a juvenile's tentacle. Spirocysts, nematocysts and gland cells are discernible in the ectoderm, and the endoderm still contains yolk granules; scale = 40 $\mu$. Labels indicate: JG = gastrovascular cavity of the juvenile; JEC = juvenile ectoderm.

FIGURE 14. Strip of mucus between ectoderms of parent and young. The juvenile has become detached; scale = 75 $\mu$. For labeling, see caption to Figures 2 and 13.

times of year must be examined histologically to ascertain a species' sexual character. There may be many more monoecious species of actinians than is commonly believed (Stephenson, 1928, pp. 91–92) because such precautions often have not been observed. Studies of anthozoan reproductive cycles also necessitate histo-

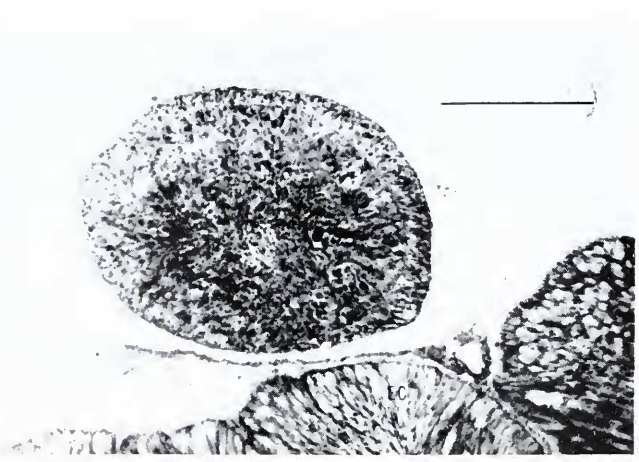

FIGURE 15. *Epiactis* embryo, slightly detached from its parent, with a band of mucus extending over it from the parent; scale = 150 $\mu$. For labeling, see caption to Figure 2.

logical examination. Since sea anemones do not have discrete gonads, I believe that a standard gonad index, as Ford (1964) used in his study of *Anthopleura elegantissima,* can be only approximately correct, at best.

Absence of vertical separation of gametes of the two sexes in hermaphroditic individuals is different from the situation in *Hydra,* where the spermary is above the ovary (Hyman, 1940), and in *Epiactis japonica,* where the situation is reversed (Uchida and Iwata, 1954).

The 12 tertiary pairs of mesenteries, some of which may be fertile, are attached to the oral end of the throat in some larger individuals, so fertile complete mesenteries can occur. Although Hand (1955) denies this, and revises Carlgren's (1949) generic definition to conform to his observations, Verrill's (1899, pp. 377–378) expanded description of *Epiactis prolifera* includes the phrase "12 or more pairs are perfect and fertile." McMurrich (1901) found that all except the first and fifth cycles of mesenteries may be fertile, and that the secondaries may also be complete. These diverse observations reflect the variability of the species, but none accurately describes the situation I found. An emendation of Hand's (1955, p. 39) revision which includes the possibility of perfect and fertile third order mesenteries would read: "At least 12 pairs of mesenteries perfect. . . . Gonads on all the stronger mesenteries, or with the perfect mesenteries *of the first two orders* sterile and the stronger (*including perfect*) mesenteries of the later orders fertile" (italicized words are my emendations).

The regularly hexamerous arrangement of the mesenteries of *Epiactis* suggests that it does not reproduce asexually. Of the 274 animals that I examined in cross-section, only one had three pairs of directive mesenteries, and one had one pair.

Oogonia begin to develop, apparently, at about the time the fourth cycle of mesenteries is completed, regardless of season. Since there are always adults of all sizes, some individuals are completing their fourth cycle of mesenteries at all times of the year.

The 72-mesentery-pair stage also appears critical in the gametogenic physiology of *E. prolifera*. A few individuals seem never to become hermaphrodites and others apparently begin spermatogenesis at all times after the formation of 72 pairs, but the change from female to hermaphrodite in most animals coincides with the formation of half the fifth cycle of mesenteries. Unlike the onset of oogenesis, however, first spermatogenesis may be related to external events. The six smallest hermaphroditic anemones were collected on four different occasions during December and March. It is unlikely that all should have been collected at about the same time of year purely by chance. There is no obvious extrinsic trigger, however, since water temperature in those four months was not equal, nor was it at an extreme, and the animals, each a different color, were from a variety of areas and intertidal heights.

The onset of oogenesis and spermatogenesis may be correlated only weakly with age of an individual. The number of mesenteries increases as the animal grows, but that is not a necessary consequence of increased age since sea anemones grow only if fed, and may even shrink if starved (Chia and Spaulding, 1972).

The data on distribution of gametes in mesenteries suggest that in hermaphrodites mesenteries of the third cycle are somewhat more male and those of the fourth cycle are somewhat more female. This is paradoxical because the tertiary mesenteries are the older ones, but eggs develop before sperm. It is, however, similar to the situation in *Epiactis japonica* (Uchida and Iwata, 1954).

The ovum of *Epiactis,* although yolky, is intermediate in size for a sea anemone (Dawydoff, 1928). The movement of the germinal vesicle to the side of the oocyte during its growth is typical of anthozoans (Chia and Rostron, 1970; Gemmill, 1920; Gohar and Roushdy, 1961; Hill, 1905; McMurrich, 1890; Nyholm, 1959) as well as other cnidarians (Beckwith, 1914; Hargitt, 1906; Hargitt, 1909). The lack of polarized orientation and synchrony of development of female gametes within each mesentery in *E. prolifera* contrasts with the situation in *Actinia equina* (Chia and Rostron, 1970). The ova of some sea anemones float when spawned (Chia and Spaulding, 1972; Gemmill, 1920, 1921; Siebert, 1973), while those of others sink (Gemmill, 1920; Nyholm, 1959). Clearly the negative buoyancy of *Epiactis* ova is absolutely necessary to the species' mode of reproduction.

Spermatogenesis, with its layering of successive stages in the follicles, is typical of most animals. *Epiactis* spermatozoa are about the same size as those of *Actinia equina* (Chia and Rostron, 1970) but considerably smaller than those of *Anthopleura stellula* (Schmidt, 1970).

As the oocytes and sperm follicles grow, the mesenteric mesogleal strand thins around them. Why they should mature within this layer, when they arise in the endoderm and must break out through it to be spawned, has never been explained. One function hypothesized for the mesoglea is storage (Chapman, 1966) and, if this were so, it might be reasonable for growing gametes to localize there where stored nutrients would be readily available. However, the Hertwigs (1879; Hertwig 1882) and Nyholm (1943) believe the trophonema has a nutritive function, and, from a structural standpoint, it does seem admirably suited to deliver nutrients from the gastrovascular cavity to the growing oocyte. Its appearance in *Epiactis* coincides with the onset of vitellogenesis, yolk granules accumulate in the region of its contact with the oocyte, it alters its form at the end of oocyte growth, and there are no nurse cells in *E. prolifera*. These findings strengthen the nutritive hypothesis. Radiotracer studies might be useful in confirming or disproving it.

If this is indeed its function, it might be expected to occur in most anthozoan species, where the growing gametes are separated from the digestive and absorptive surface, but few studies of anthozoan reproduction mention it. It would also leave unexplained the reason for the gametes' migration into the mesoglea to mature.

It has been suggested that sperm might reach an oocyte through the channel of the trophonema. Because of the likelihood of post-spawning fertilization (see below), and because the structure appears so early in the oogenic process and the channel closes or disappears entirely at just about the time the egg cell finishes growing and might be ready to be fertilized, this is unlikely. [Development of the trophonema in *Epiactis* is the same as that described by Nyholm (1943) for *Cerianthus lloydii*.]

When and where reduction division and fertilization (not necessarily in that order) occur in *E. prolifera* are unknown, but I infer that neither happens before spawning because there is no division of the eggs while in the mesenteries, unlike in *Actinia equina* (Chia and Rostron, 1970), and the oocyte shown escaping from the mesentery in Figure 8 still has a large germinal vesicle. All large oocytes in the mesoglea have a conspicuous nucleus, but those in the throat of an animal fixed while spawning have no detectable nucleus, as is true of other species of sea anemones in which fertilization does not occur until after the ova have been spawned (Chia and Spaulding, 1972; Gemmill, 1921; McMurrich, 1890; Nyholm, 1959).

Although gametes in all stages of maturity are present throughout the year, external stimuli are almost certainly required for spawning, since it is at least sometimes an epidemic phenomenon. Spawning, either epidemic or not, occurs during much of the year, for at least 27% of the rocky intertidal population was brooding at any time, and spawning or recently-spawned individuals were found during seven months of the year (February through July, and October) (Dunn, 1972). Thus *E. prolifera* resembles many other temperate species that produce large yolky eggs or brood, in having an extended breeding season (Giese, 1959; Thorson, 1936).

Because it is gynodioecious, there is certainly some outbreeding in *Epiactis*, but larger individuals might be self-fertile since their sperm and ova appear to be ripe simultaneously. This is unlikely, however, for when such hermaphrodites are studied in detail, it is usually found that while self-fertilization may be possible, there are mechanisms to prevent it (*e.g.* Silén, 1966).

The oocytes (or ova?) found in the gullets of juveniles, and the earliest stages attached to parent ectoderm have no visible nucleus, so it is uncertain whether they are fertilized by this time either. Perhaps eggs adhere to parent ectoderm and are fertilized there (maturation divisions may even occur there), but in the event that development does not proceed, the ectoderm releases the sphere. This would be adaptive since it would maximize the productive use of the limited brood area. It might also account for the observation that the oocytes that were spawned in the laboratory and became attached to the parent column had disappeared nine days later. Since the adult was alone in a finger bowl, the spheres were probably unfertilized (unless self-fertilization or parthenogenesis can occur). Or perhaps non-viable oocytes, ova and zygotes simply deteriorate and are washed away.

The finding of freshly-spawned oocytes in the thorats of (sterile) juveniles supports the hypothesis that maximum brood size is limited by the amount of

brood space (Dunn, 1972). As eggs spawned by a parent already brooding fairly large young tumble down its column, some may fall onto empty spots of the brood area, keeping it filled, some may fall onto the substrate, but others may fall onto the juveniles' tentacles and provoke a feeding response. They might not be digested by the juveniles, however, since adult specimens of *Epiactis* will ingest other *Epiactis* one-tenth their size, but within several hours will regurgitate them unharmed (Lenhoff, 1965). Should the oocytes in the juveniles' gullets be egested and fall onto unoccupied space in the parental brood area, it is possible that they would develop into juvenile anemones. In fact, since not all eggs that are spawned adhere immediately to the parent ectoderm, this may be a way of reducing loss of them.

It might be hypothesized, by analogy with *Actinia equina* (Chia and Rostron, 1970), that young *Epiactis* are spawned into the plankton, or remain briefly on the parent column before entering the plankton, only later settling on "foster parents." The facts that no *E. prolifera* brooding young was sterile, that all stages of embryogenesis were found among young being brooded, and that parent and juvenile coloration are so similar (Dunn, 1972) make it virtually certain that each animal carries its own offspring.

Dawydoff (1928) states that as many as 32 daughter nuclei may form in anthozoan zygotes before cellularization begins, but it appears that in *Epiactis* the number is greater. Meroblastic cleavage occurs in other anemones, but whereas an ectodermal epithelium forms around a mass of uncleaved yolk in *Actinia bermudensis* (Cary, 1910), *Tealia crassicornis* (Chia and Spaulding, 1972) and *Stomphia didemon* (Siebert, 1973), in *E. prolifera* the endoderm is composed of a few large cells from the onset of cytokinesis. In none of these species does a blastocoel develop.

Johnson and Snook (1927) and MacGinitie and MacGinitie (1968) state that the larvae of *E. prolifera* develop to a considerably advanced stage in the parent enteron, then make their way to the outside where they attach to the parent. They may have been influenced in their incorrect interpretation of the sequence of events by Carlgren (1901), who found no juvenile *Epiactis* without pharynx and mesenteries being brooded, so concluded that none in less developed stages ever occurs on the outside.

Although there are no distinct brood-pits in *E. prolifera*, the ectoderm around each embryo enfolds it, thereby preventing its falling off, but it is unlikely that the ectodermal response is sufficiently rapid to catch a falling sphere as it tumbles down the column. The freshly-spawned spheres probably first adhere in another manner. Mucoprotein secreted by gland cells in the parent ectoderm may be the original cause of adhesion of the spheres. In later stages, mucus covers the embryos, which aids in holding them on the column (Fig. 15), and this is supported by the observation that if a juvenile is pulled off its parent, a band of stringy mucus encircling the brood area becomes apparent. When it is carefully peeled off the adult, some of the smallest juveniles come with it. In juveniles, mucus acts as an adhesive between the ectoderms of parent and offspring (Fig. 14). Uchida and Iwata (1954) believe that mucus is very important in adhesion of young *Epiactis japonica* to the columns of their parents.

Large atrichous nematocysts, heretofore reported in *E. prolifera* only by K. W. England [British Museum (Natural History), personal communication to C.

Hand] occur in the brood area. In the population I studied, they measure 23.1–29.4 × 2.1–4.2 $\mu$ (N = 26) in fixed animals, and 24.0–31.7 × 2.9–4.3 $\mu$ (N = 20) in fresh smears. I did not find them in small, non-brooding individuals. The restriction of these nematocysts to the lower column of larger adults suggests that they might help to hold the young on the parent column, perhaps being particularly important in initiating adhesion.

It is likely that the parent remains the active partner in maintaining the relationship for its entire duration because even relatively large juveniles have no mucoprotein gland cells nor nematocysts in their columns. When a juvenile finally leaves, it is probably because it has become sufficiently strong to overcome the adhesive forces of the parent or because it is pushed off by growing siblings (Dunn, 1972).

The intimate association between parent and young suggests a nutritive relationship, a possibility considered likely by Verrill (1869) in his original description of *E. prolifera*. However, like other brooders (Thorson, 1950), *Epiactis* produces few large yolky eggs relative to nonbrooding species. Thus the parent has already provided the developing offspring with an energy reserve, and further contribution to its nourishment would appear to be redundant and probably unnecessary (*cf.* Silén, 1945). Although it seems unlikely, experiments using radioactive food could be done to determine whether translocation of nutrients from parent to young does occur. *Epiactis* not only has large yolky eggs, but even tiny juveniles are capable of capturing small prey. They may derive a nutritional advantage from their close proximity to their parents, since they do obtain bits of the food held by their parents or contained in the egestate of the parents (Dunn, 1972). Thus food items which might be too large or strong for them to obtain by themselves are available for sharing by the young. The range in size and variety of potential prey is therefore probably greater for attached young than it would be for isolated animals of the same size.

I thank Dr. Cadet Hand, who first pointed out to me the lack of knowledge about external brooding in actinians and the unusually favorable opportunity for its study in *Epiactis prolifera*, for his help and advice; the faculty, staff and students of the Zoology Department of the University of California/Berkeley and of the Bodega Marine Laboratory for their aid and suggestions, in particular Dr. Ralph I. Smith; the late Dr. J. Ralph Andy, Director of the Hooper Foundation, University of California/San Francisco, for use of facilities in the Foundation; Mr. Stanley Watkins, for excellent instruction in microtechnique; Mr. Charles Fautin, for field assistance; two anonymous reviewers; and, most gratefully, my husband, Fred.

## Summary

1. *Epiactis prolifera* is a gynodioecious hermaphrodite, most of the intermediate-size individuals being female and most of the larger ones hermaphroditic. The population studied lacked purely male individuals.

2. Fertile animals may be found not brooding young, but all brooding individuals examined were fertile. Both females and hermaphrodites brood.

3. Gametogenesis proceeds in characteristic fashion, although oogenesis is accompanied by the formation of a trophonema between the oocyte and edge of the mesentery. All stages of both types of gametes were present throughout the year.

4. The zygotic nucleus divides a number of times and the resulting nuclei segregate into two layers before cytokinesis occurs. Mesenteries form while endodermal cells are still yolk-laden, and before it is all absorbed, tentacles with functional nematocysts and gland cells develop. Thus attached juveniles are capable of capturing prey.

5. Mucus and perhaps nematocysts, both of parental origin, are probably responsible for adhesion of the young to the parent's column. Enfolding by the parent ectoderm may also play a role in the early stages.

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